WHAT IS CLAIMED IS:

1. A method of constructing a recombinant polynucleotide comprising the steps of

(a) generating a mixture containing linear polynucleotide fragments derived from a parent polynucleotide, wherein each polynucleotide fragment (i) is double-stranded, with the exception of at least one single-stranded sticky end, (ii) has a first single-stranded sticky end at the first end and a second single-stranded sticky end at the second end, such that (iii) in an average of at most 1 in 256 of linear polynucleotide fragments generated, the first single-stranded sticky end is not complementary to the second single-stranded sticky end;

(b) introducing to the mixture at least one additional polynucleotide fragment, which (i) is linear, (ii) is double-stranded across, with the exception of a single-stranded sticky end, (iii) has a first single-stranded sticky end at the first end and a second single-stranded sticky end at the second end, such that (iv) in an average of at most 1 in 256 of linear polynucleotide fragments generated, the first single-stranded sticky end is not complementary to the second single-stranded sticky end to the fragments, and (v) the first and second single-stranded sticky ends of the additional polynucleotide are compatible to either the first single-stranded sticky end or the second single-stranded sticky end of one or more linear polynucleotide fragments of the mixture; and

(c) ligating the polynucleotide fragments and the said additional polynucleotide together to produce a recombinant polynucleotide.

2. The method according to claim 1 wherein the parent polynucleotide is viral DNA

3. The method according to claim 1 wherein the parent polynucleotide is bacterial DNA.

4. The method according to claim 1 wherein the parent polynucleotide is a DNA sequence acquired from plants.
5. The method according to claim 1 wherein the parent polynucleotide is an artificially produced DNA sequence.
6. The method according to claim 1 wherein the parent polynucleotide is a naturally occurring DNA sequence.
7. The method according to any one of claims 1-6, wherein the mixture containing linear polynucleotide fragments is generated by cutting the parent polynucleotide with an asymmetric endonuclease.
8. The method according to claim 7 wherein the asymmetric endonuclease is selected from the group consisting of AccB7I, AhdI, AleI, AlwI, Alw26I, AlwNI, BbsI, BbvI, BccI, BceAI, BciVI, BfuAI, BglI, BlpI, BpmI, Bpu10I, BpuEI, BsaI, BsaBI, BsaJI, BsaMI, BsaRI, BsgI, BslI, BsmI, BsmAI, BsmBI, BsmFI, BspCNI, BsrI, BsrDI, BsrSI, BssKI, Bst71, BstAPI, BstEII, BstF5I, BstXI, Bsu36I, BtsI, Cac8I, DdeI, DraIII, EarI, EciI, EclHKI, EcoNI, FauI, Fnu4HI, FokI, HgaI, HinfI, HphI, Hpy188I, Hpy188III, MboII, MlyI, MnlI, MsII, MwoI, NlaIV, PflFI, PflMI, PleI, PshAI, SapI, Sau96I, ScrFI, SfaNI, SfiI, StyD4I, TspRI, Tth111I, XcmI, and XmnI.
9. The method according to claim 7 or claim 8 wherein the asymmetric endonuclease is BstXI.
10. The method of claim 1 where the additional polynucleotide is an artificial chromosome.
11. The method according to claim 9 or claim 10 wherein the artificial chromosome is a bacterial artificial chromosome.

12. The method according to claim 9 or claim 10 wherein the artificial chromosome is a mammalian artificial chromosome.

13. The method according to claim 9 or claim 10 wherein the artificial chromosome is a yeast artificial chromosome.

14. The method according to any one of claims 1 though 9 wherein the parent polynucleotide is a viral genome or a fragment thereof.

15. The method of claim 14 wherein the recombinant polynucleotide is selected from the group consisting of gene therapy vector, vaccine, a plant vector useful in the production of beneficial products, and recombinant polypeptide production vector.

16. The method of claim 14 wherein the recombinant polynucleotide is a gene therapy vector and the parent polynucleotide is an adenoviral vector.

17. A kit comprising (a) at least one polynucleotide fragment having a first sticky end and a second sticky end, wherein the first sticky end is non-complementary to the second sticky end, (b) a ligase, (c) a buffer and (d) an oligonucleotide.

18. The kit according to claim 17 comprising BstXI-generated polynucleotide fragments of an adenovirus genome and an oligonucleotide, which comprises a BstXI restriction site as set forth in SEQ ID NO:1 and an adenovirus sequence.

19. The kit according to claim 18 wherein the kit is useful in the production of a recombinant adenoviral-based vector comprising a modified polynucleotide sequence.

20. The kit according to claim 18 wherein the adenoviral-based vector is a gene-therapy vector and the modified polynucleotide sequence encodes a therapeutic polypeptide.